

**AMENDMENT AND REQUEST FOR RECONSIDERATION  
UNDER 37 C.F.R. § 1.116  
U.S. Appln. No.: 09/185,607**

expressing SP2/0 cells that are transfected with a vector encoding an antibody having one or more N-glycosylation sites in the CH1 or V $\kappa$  domain in a culture medium comprising a ketone derivative of a saccharide or biosynthetic saccharide precursor so that they produce a glycosylated antibody having a reactive ketone group on the glycosylated site, and

fragmenting the resulting glycosylated antibody into] to produce a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site.

8. (Twice Amended) A method of making an immunoconjugate comprising a glycosylated antibody conjugated to an agent through its glycosylated site, comprising:

reacting a glycosylated antibody produced according to claim 1 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, thereby conjugating said glycosylated antibody to said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

16. (Twice Amended) A method of making an immunoconjugate comprising a glycosylated antigen-binding antibody fragment conjugated to an agent through the glycosylated site, comprising:

reacting a glycosylated antibody fragment produced according to claim 6 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, thereby conjugating said glycosylated antibody fragment to said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

19. (Twice Amended) A glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, wherein said glycosylated site is in the V $\kappa$  or CH1 domain, and wherein the reactive ketone group is not introduced by oxidation.

**AMENDMENT AND REQUEST FOR RECONSIDERATION**  
**UNDER 37 C.F.R. § 1.116**  
**U.S. Appl. No.: 09/185,607**

22. (Twice Amended) An immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an agent through the glycosylated site, wherein said glycosylated site is in the V $\kappa$  or CH1 domain, and wherein the agent is conjugated to a reactive ketone group on the glycosylated site that is not introduced by oxidation.

30. (Twice Amended) A method of targeting an active agent to an *in vivo* target site comprising administering an immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an active agent through a reactive ketone group on a glycosylated HCN1, HCN5 or V $\kappa$ -N glycosylation site and not as a conjugate to an oxidized sugar.

38. (Amended) A method of making a glycosylated antibody having a reactive ketone group on the glycosylated site, comprising:

expressing SP2/0 cells that are transfected with a vector encoding an antibody having a HCN1, HCN5 or V $\kappa$  N-glycosylation site in a culture medium comprising a ketone derivative of a saccharide or biosynthetic saccharide precursor, so that they produce an N-glycosylated antibody having a reactive ketone group on the glycosylated site.

40. (Amended) A method according to claim 38, wherein the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose.

41. (Amended) A method making a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, comprising:

expressing SP2/0 cells that are transfected with a vector encoding an antibody having a HCN1, HCN5 or V $\kappa$  N-glycosylation site in a culture medium comprising a ketone derivative of a saccharide or biosynthetic saccharide precursor so that they produce

**AMENDMENT AND REQUEST FOR RECONSIDERATION  
UNDER 37 C.F.R. § 1.116  
U.S. Appl. No.: 09/185,607**

fragmenting the resulting glycosylated antibody into a glycosylated antigen-binding antibody fragment having a reactive ketone group on the glycosylated site.

43. (Amended) A method according to claim 41, wherein the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose.

44. (Amended) A method of making an immunoconjugate comprising a glycosylated antibody conjugated to an agent through its glycosylated site, comprising:

reacting a glycosylated antibody according to claim 38 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, thereby conjugating said glycosylated antibody to said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

46. (Amended) A method according to claim 44, wherein the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose.

47. (Amended) A method of making an immunoconjugate comprising a glycosylated antigen-binding antibody fragment conjugated to an agent through the glycosylated site, comprising:

reacting a glycosylated antibody fragment according to claim 41 with an agent comprising a ketone-reactive group selected from the group consisting of hydrazides, hydrazines, hydroxylamines, and thiosemicarbazides, thereby conjugating said glycosylated antibody fragment to said agent through the reactive ketone group on its glycosylated site, wherein the reactive ketone group is not introduced by oxidation.

49. (Amended) A method according to claim 47, wherein the ketone derivative of the saccharide or biosynthetic saccharide precursor is selected from the group consisting of N-levulinoyl mannosamine and N-levulinoyl fucose.

**AMENDMENT AND REQUEST FOR RECONSIDERATION**  
**UNDER 37 C.F.R. § 1.116**  
**U.S. Appln. No.: 09/185,607**

53. (Amended) A glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on a glycosylated site, wherein said glycosylated site is selected from the group consisting of HCN1, HCN5 and V $\kappa$ -N, and wherein the reactive ketone group is not introduced by oxidation.

54. (Amended) An immunoconjugate comprising a glycosylated antibody or antigen-binding antibody fragment conjugated to an agent through a reactive ketone on a glycosylated site, wherein said glycosylated site is selected from the group consisting of HCN1, HCN5 and V $\kappa$ -N, and wherein the reactive ketone group is not introduced by oxidation.

55. (Amended) A glycosylated antibody having a reactive ketone group on a glycosylated site, prepared by a method as recited in claim 1, wherein the reactive ketone group is not introduced by oxidation.